DOCKET NO.: ISIS-3105 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#21

In re application of:

Teng and Hardee

Serial No.: **09/108,673** Group Art Unit: **1636**

Filed: **July 1, 1998** Examiner: **W. Sandals**

For: **COMPOSITIONS AND METHODS FOR THE DELIVERY OF OLIGONUCLEOTIDES VIA THE ALIMENTARY CANAL**

Assistant Commissioner
for Patents,
Washington, D.C. 20231

Dear Sir:

Declaration of Dr. Greg Hardee/Ching-Leou Teng

I, Greg Hardee/Ching-Leou Teng, hereby declare as follows:

1. I am a named co-inventor, along with Greg Hardee/Ching-Leou Teng, of U.S. application serial number 09/108,673 filed Jul1 1, 1998, entitled "Compositions And Methods For The Delivery Of Oligonucleotides Via The Alimentary Canal."

2. I have performed, supervised, or instructed the performance of the experiments described below.

3. In order to evaluate the capacity of fatty acids to enhance penetration of a nucleic acid across the alimentary canal of an animal, three formulations of ISIS 2302 (an oligonucleotide directed against intracellular adhesion molecule 1 (ICAM-1); SEQ ID NO:1 in the above-identified application) were prepared. Formulation 1 comprises 1 mg/ml of ISIS 2302 oligonucleotide and 1%

w/v laurate (Na salt of lauric acid, Sigma Chemical Company, St. Louis, MO). Formulation 2 comprises 1 mg/ml of ISIS 2302 oligonucleotide and 1% w/v caprate (Na salt of capric acid, Sigma Chemical Company). Formulation 3 comprises 1 mg/ml of ISIS 2302 oligonucleotide, 0.5% w/v laurate and 0.5% w/v caprate. The formulations were prepared as follows. 1) buffer: in a volumetric flask, the following were combined: 14.33 g dibasic sodium phosphate, heptahydrate (U.S.P.); 1.73 g monobasic sodium phosphate, monohydrate (U.S.P.); and 4.4 g sodium chloride (U.S.P.). The volume was brought to 1 liter with purified, deionized water. The pH of the buffer was 7.4 and had an osmolality of approximately 290 mOsm/kg. 2) ISIS 2302 Stock Solution: in 30 ml of purified, deionized water, 10 g of pure, anhydrous ISIS 2302 was dissolved. The solution was adjusted to pH 7.4 with 1.0 N NaOH. The volume was adjusted to 50 ml with purified water to yield a final concentration of 200 mg/ml of oligonucleotide ISIS 2302. 3) Formulation 1: 500 mg of sodium laurate was transferred to a 50 ml volumetric flask containing about 40 ml buffer. An aliquot of 250 µl of ISIS 2302 solution was then added to the buffer solution. The solution was titrated to pH 7.4 with 0.1 N HCl, and the volume of the solution was adjusted to 50 ml with buffer. 4) Formulation 2: 500 mg of sodium caprate was transferred to a 50 ml volumetric flask containing about 40 ml buffer. An aliquot of 250 µl of ISIS 2302 solution (200 mg/ml) was added to the buffer solution. The solution was titrated to about pH 7.7 with 0.1 N HCl, and the volume of the solution was adjusted to 50 ml with buffer. 5) Formulation 3: 250 mg of sodium laurate and 250 mg of sodium caprate were transferred to a 50 ml volumetric flask containing about 40 ml buffer. An aliquot of 250 µl of ISIS 2302 solution was then added to the buffer solution. The solution was titrated to pH 7.4 with 0.1 N HCl, and the volume of the solution was adjusted to 50 ml with buffer.

4. The Formulations described in paragraph 3 were evaluated by *in situ* perfusion of rat ileum as follows. *In situ* perfusion of rat ileum was performed essentially according to the procedure of Komiya *et al.*, *Int. J. Pharmaceut.*, **1980**, *4*, 249. Specifically, male Sprague Dawley rats weighing 250-300 g were used. After overnight fasting, the rats were anesthetized with 5% pentobarbital (50 mg/kg) by intraperitoneal injection. After a midline abdominal incision was made, the small intestine was taken out and the ileum section was located. An incision was made at each end of a 20 cm ileum

segment. The ileum segment was laid out in a uniform multiple-S arrangement with 3 bends. The luminal contents of the section were flushed with buffer and a 10 cm piece of silicon rubber tubing was inserted into the intestinal lumen at each incision and ligated with 3-0 silk suture. The proximal end tubing was connected to a 30 ml syringe containing a oligonucleotide formulation. The formulation was perfused through the intestinal segment by using Sage model 365 syringe pump at 0.125 ml/minute. The outflow solution was collected in a 2 ml centrifuge tube over 5 minute intervals for 80 minutes. At the end of perfusion study, an aliquot of 0.3 ml blood sample was collected from the portal vein. ISIS 2302 concentration in the solution before and after passing through a 20 cm ileum segment was analyzed by high pressure liquid chromatography (HPLC) while the plasma samples were analyzed by capillary electrophoresis (CE). In most cases, tritium labeled ISIS 2302 was used as a tracer and the radioactivity of solution was measured by liquid scintillation counter. The amount of the drug absorbed from the ileum was calculated by dividing the concentration from the average of last six outflow samples (steady state) to that of the inflow sample. No significant amount (i.e., about 0%) of ISIS 2302 was absorbed at steady state when a control formulation (i.e., one lacking any penetration enhancers) was used. In contrast, approximately 5% of ISIS 2302 was absorbed at steady state with a 20 cm ileum segment when Formulations 1 or 2 were perfused. The absorption increased to 15% when Formulation 3 was used. The amounts absorbed were reflected in blood samples collected from the portal veins of the rats. The plasma concentration of ISIS 2302 was 0.29 μg/ml with Formulation 1 and increased to 2.83 μg/ml with Formulation 3. Thus, a composition having at least two fatty acids enhances penetration of an oligonucleotide across the alimentary canal of an animal.

5. In order to evaluate the combination of two or more different types of penetration enhancers, three formulations of ISIS 15839 (an oligonucleotide directed against the same sequence of ICAM-1 as ISIS 2302 except that ISIS 15839 is a fully 5-methylcytidine (m5c) "hemimer" with the eight 3' terminal nucleotides modified by 2'-methoxyethoxy). Dogs were "ported" with intestinal access catheters through which formulated drug formulations were introduced into various areas of the gut. Target areas included the proximal jejunum and distal ilium or the ileocecal junction. ISIS

15839 was administered intrajejunally to "ported" dogs at oligonucleotide doses of 10 mg/kg with or without penetration enhancers. Specifically, an aliquot of 20 mg/ml drug solution was injected into a subcutaneous port catheter connected to the proximal jejunum. A bile salt (Na salt of chenodeoxycholic acid, CDCA) was used alone or in combination with fatty acids (2% CDCA, 4% Na caprate, 4% Na laurate). Blood samples were collected from the femoral vein for up to 6 hours and evaluated for the presence and concentration of oligonucleotides by HPLC. Percent bioavailability was calculated. Delivery of ISIS 15839 in water or saline without a penetration enhancer resulted in 1.5% bioavailability. Delivery of ISIS 15839 with 2% CDCA resulted in 4.4% bioavailability. Delivery of ISIS 15839 with 4% Na caprate and 4% Na laurate resulted in 2.5% bioavailability. Delivery of ISIS 15839 with 2% CDCA, 4% Na caprate and 4% Na laurate resulted in 18.0% bioavailability. Thus, a composition having at least two fatty acids and a bile salt enhances penetration of an oligonucleotide across the alimentary canal of an animal.

6. I declare that all statements made herein are of my own knowledge true and statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Dr. Greg Hardee/Ching-Leou Teng

7/11/00

Date

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Dr. Greg Hardee

12/15/00
Date

Ching-Leou Teng
Dr. Ching-Leou Teng

12/19/00
Date